# STANDARDIZATION OF A METHOD FOR IDENTIFICATION OF ELECAMPANE SESQUITERPENE LACTONES

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**Introduction**. A wide use of elecampane, *Inula helenium* L. (*Asteraceae*), in both, folk and formal medicine, is explained by variety of its chemical composition. The main action, owing to which elecampane is used in folk medicine, is expectorant. Indications for medical use of elecampane rhizomes with roots include diseases of the respiratory and gastrointestinal tracts [1]. An anti-ulcer drug product based on sesquiterpene lactones (SQTL) elecampane, Alanton, was widely known in 1980s [2].

Rhizomes and roots of elecampane contain an essential oil, the content of which reaches 4%; the composition of the oil includes a mixture of SQTL such as Eudesman type. The main components of this mixture are alantolactone (0.5-2.0%), isoalantolactone (1.0-2.7%), and their hydrogenated derivatives: dihydroalantolactone, dihydroisoalantolactone, tetrahydroalantolactone, etc (Fig.1) [3,4]. These compounds seem to be responsible for pharmacological action of elecampane-based preparations.

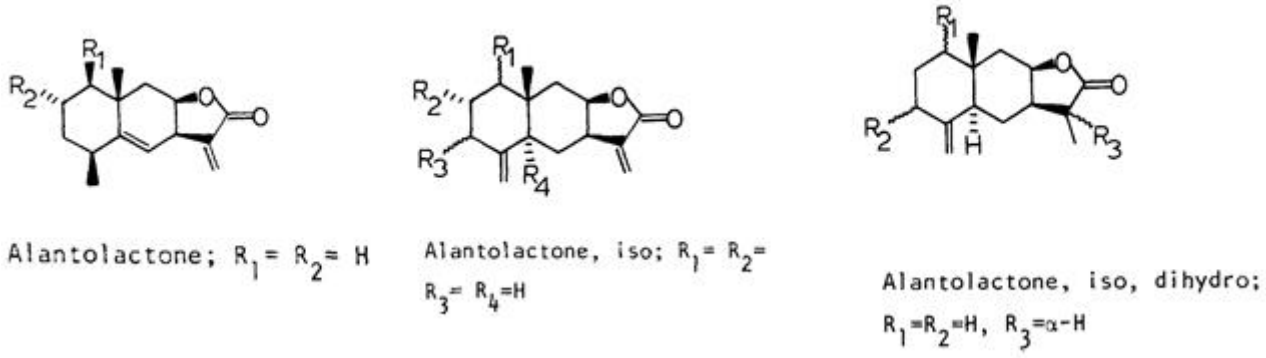


Figure 1. Some of the main biological active constituents of elecampane

Herbal drug (HD) from elecampane is rhizomes and roots (Fig. 2). This type of HD is described in many pharmacopoeias – the State Pharmacopoeia of the USSR 11 ed. (GF XI) [5], the British Herbal Pharmacopeia (BHP) [6], the French Pharmacopoeia (Ph. Fr.) [7], the Chinese Pharmacopoeia 2005 (CPh 2005) [8], Ayurvedic Pharmacopoeia of India (APhI) [9], and the State Pharmacopoeia of the Republic of Belarus (GF RB) [10]. Currently, there is no monograph on this type of HD in the State Pharmacopoeia of Ukraine (SPhU) [11], therefore research related to its development is relevant.

In accordance with the concept of introducing a monograph on HDs in the SPhU, before the development of a national monograph, the quality of domestic HDs is regulated by the requirements of the relevant article of GF XI. In the article GF XI "Rhizomes and roots of elecampane", HD is standardized by macro and microscopic features, a qualitative reaction for inulin, moisture content, ash content and foreign matter. The mandatory identification test for HDs by the thin-layer chromatography (TLC) method in accordance with the requirements of SPhU is absent in this article, therefore it is necessary to develop a procedure for identification of the main biological active substances (BAS) of elecampane by TLC-method. As an object of such studies, we have chosen SQTL elecampane as markers of this species and, in some cases, genus.

## Materials and Methods

The rhizomes and roots of *Inula helenium* L. (Asteraceae) were obtained from various pharmaceutical enterprises of Ukraine during 2016-2017. The identification and authentication of the plant material was carried out by Assosiate Prof. Vovk A.G. from the department «Experimental support the elaboration of monographs on herbal drugs» of the SE «Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines» (Kharkov, Ukraine).

The following equipment was used: Silica gel 60 $F_{254}$ TLC plates (20x20 $cm^2$, 0.25 mm), Merck (Germany); Ultrasonic bath SUPER RK100H «Bandelin», (Germany); Glass vertical chamber; Automatic spraying device ChromaJet DS20.

As standard substances have been used alantolactone, isoalantolactone and dihydroisoalantolactone (<95%) - pharmacopoeial reference standards of the State Pharmacopoeia of Ukraine.

Solvents (methanol, ethyl acetate, toluene, water) and chemicals (sulfuric acid, acetic acid, anise aldehyde, silver nitrate) used in the experiments were of analytical grade.

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**Figure 2. Elecampane – dried drug substance (rhizome)**

**Results and discussion**

When developing the procedure, the conditions for conducting BAS identification by physical and chemical methods described in the normative documents listed above have been previously studied. It has been found that in the monograph of the GF RB, as well as in the GF XI article, there is no identification by TLC. Identification technique by TLC for methanol extract of elecampane with description the Rf bands and their coloring after processing the chromatogram with vanillin solution is described in the monographs of APhI and BHP. The disadvantage of these methods is the absence of marker substances with respect to which it is possible to describe reliably the position of the zones in the chromatogram of the test solution.

The TLC method is also described for identification of SQTL elecampane in the monograph of the Ph. Fr., according to which a solution of the essential oil (after preliminary distillation from the herbal material) in hexane is used as test solution. Isopropyl ether-hexane mixture (20:80) is used as mobile phase, the detection is carried out after treatment the plate with vanillin solution and followed by heating, and the Rf zone of alantolactone is described in the chromatogram. The disadvantages of this technique are rather labor-intensive procedure for preparing of elecampane test solution, the absence of marker substances, and description of only one zone of alantolactone, in spite of presence of sufficient amounts of its isomer (in double bond position), isoalantolactone in elecampane.

The TLC-method is described in the monograph of the CPh 2005 with the use of methanol extract from elecampane and two marker substances: alantolactone and isoalantolactone. Silica gel G mixed with 0.25% silver nitrate solution as a sorbent, petroleum ether-benzene-ethyl acetate mixture (5:1:1) as mobile phase are used in the procedure; the detection is carried out after treatment the plate with anise aldehyde solution and followed by heating. Two zones are described in the chromatogram of the test solution, which correspond in their position and color to those of the reference substances. This technique benefits significantly compared to those listed above, its only shortcoming is self-applying of a sorbent layer to the plate.

It should be noted that silver ions are widely used in chromatographic techniques (HPLC, TLC, column chromatography) to improve the separation of fatty acids, lipids, including positional isomers, which are alantolactone and isoalantolactone. Application of this method was based on those differences in the number, geometrical configuration and position of unsaturated C-C bonds in molecules. Unsaturated C-C bond usually act as π-donors, while silver ion – as π-acceptors thus causing chromatographic behavior for those contain fever π-donors or do not contain π-donors in molecules to be changed [12,13].

A procedure for identification of the SQTL elecampane by TLC method for national monograph of the SPhU "Elecampane roots and rhizomes" has been developed as a result of analysis of the methods described, a large-volume experiment with comprehensive study of SQTL elecampane carried out by the authors back in 1990s [4], and also taking into account experience of developing unified TLC techniques for monographs of the SPhU [14].

The development of a procedure for identification was carried out in conjunction with its validation in accordance with the requirements of SphU according to the following scheme:

1) the choice of stationary phase (examination of plates with a thin layer of silica gel treated with 1, 3, 5, 7% (m/v) solutions of silver nitrate) (Fig. 3);

2) the choice of mobile phase (review of unified phases for chromatography of terpenoids);

3) the choice of concentration for markers-substances (study of 0.05, 0.1, 0.2% methanol solutions of alantolactone, iso-alantolactone, dihydroiso-alantolactone and SQTL mixture for preparation of the reference solution);

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4) the choice of the method for test solution preparation (study of extraction of herbal material with methanol, followed by concentration of the extract to the ratio of HD-test solution 1: 2, 1: 5, 1: 8); the choice of application volume of the test solution (Fig. 4);

5) the choice of the detection method (review of unified reagents and/or solutions for derivatization of chromatograms).

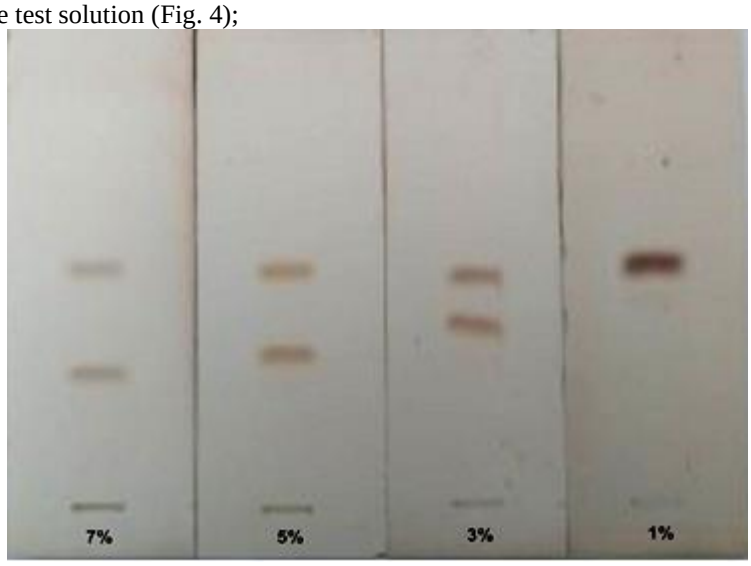


**Figure 3. Chromatograms obtained by studying the effect of the solution concentration (1-7%, m/v) of silver nitrate for treatment of a silica gel layer on its ability to separate isomers - alantolactone / iso-alantolactone.**

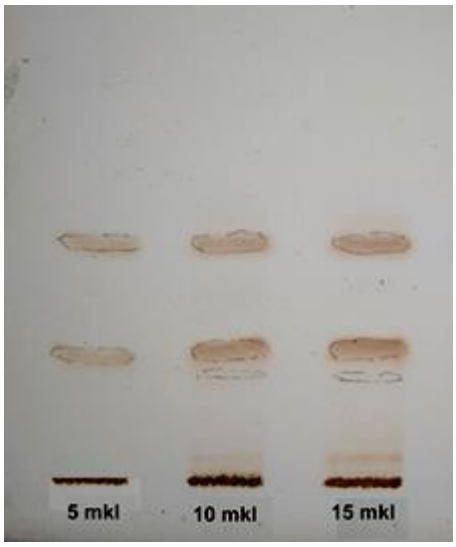


**Figure 4. A chromatogram obtained by choosing the application volume of the test solution prepared from elecampane roots and rhizomes.**

As a result, the following conditions for identification have been chosen:

1. *Plate*. *TLC silica gel $F_{254}$ P* (Merck) on support of glass, which is impregnated by spraying with 5% solution (m/v) of *silver nitrate P* using 10 mL for a 200 $mm^2$ square plate, is dried in a warm air stream and heated at 105 °C for 10 min;

2. *Mobile phase*. *Toluene P – ethyl acetate P* (9:1); it is unified because it is used in several monographs of the SPhU for analysis of HDs containing terpenoids;

3. *Reference solution*. Solution *CRS SPhU mixture alantolactone and iso-alantolactone* in *methanol P* (2 mg/ 2 mL);

4. *Test solution*. To 1.0 g of the powdered herbal drug (500) (*2.9.12*) add 20 mL of *methanol P*, sonificate for 30 min and filter. Evaporate under vacuum and dilute to 5.0 mL with *methanol P*;

5. *Application*. 15 µl as bands of 10 mm;

6. *Detection*. Treat with *anisaldehyde solution R* and heat at 100 °C for 5 min; examine in daylight; the procedure is also unified.

Fig. 5 shows the type of chromatograms obtained under the conditions developed for analysis of various batches of domestic production elecampane. As it can be seen from Fig.5, characteristic zones appear in all chromatograms of the samples: two intense brown zones corresponding in position and color to those of alantolactone (zone with higher $R_f$) and isoalantolactone (zone with lower $R_f$) in the chromatogram of the reference solution; in addition, in all chromatograms of the test solutions, there is a weak brown zone located below the

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isoalantolactone zone and corresponds to that of a standard sample of dihydroisoalantolactone.

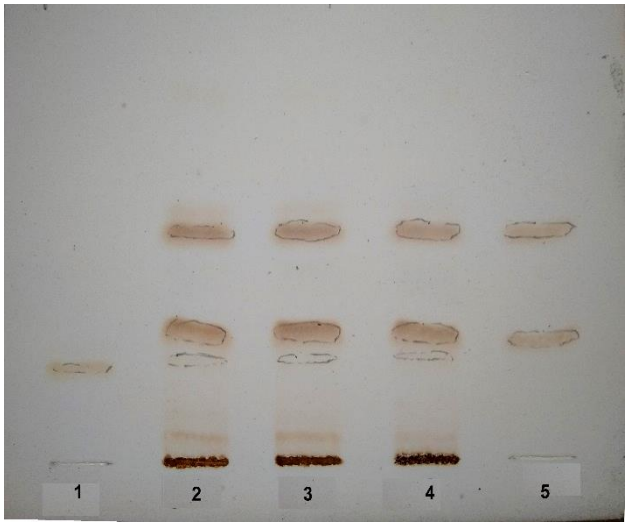


**Figure 5. The type of chromatograms obtained by identification of various samples of elecampane roots and rhizomes under conditions of the procedure developed: 1- solution of *CRS SPhU of dihydroisoalantolactone*, 2-4 - test solutions of various HD samples, and 5- solution of *CRS SPhU of alantolactone and iso-alantolactone.***

**Conclusion**. A procedure for identification of elecampane by TLC method for the national monograph of the SPhU "Elecampane roots and rhizomes" has been developed.

It allows to identify such biologically active substances of the elecampane, as sesquiterpene lactones, which are markers of this species.

The developed chromatographic conditions allow to reliably chromatographically identify HD of elecampane in the presence on chromatograms of 3 zones of lactones – alantolactone, isoalantolactone and dihydroisoalantolactone.

**Standardization of a method for identification of elecampane sesquiterpene lactones**

**Elina Kotova, Semen Kotov, Andrey Kotov**

**Introduction** A wide use of elecampane, *Inula helenium* L. (*Asteraceae*), in both, folk and formal medicine, is explained by variety of its chemical composition. Rhizomes and roots of elecampane contain an essential oil, the content of which reaches 4%; the composition of the oil includes a mixture of sesquiterpene lactones. The main components of this mixture are alantolactone (0.5-2.0%), isoalantolactone (1.0-2.7%), and their hydrogenated derivatives: dihydroalantolactone, dihydroisoalantolactone, tetrahydroalantolactone, etc. These compounds seem to be responsible for pharmacological action of elecampane-based preparations. Herbal drug (HD) from elecampane is rhizomes and roots. There is no monograph on this type of HD in the State Pharmacopoeia of Ukraine (SPhU), therefore research related to its development is relevant. The mandatory identification test for HDs by the thin-layer chromatography (TLC) method in accordance with the requirements of SPhU is absent in the article GF XI "Rhizomes and roots of elecampane", therefore it is necessary to develop a procedure for identification of the main biological active substances (BAS) of elecampane by TLC-method.

**Materials and Methods.** The rhizomes and roots of *Inula helenium* L. (Asteraceae) were obtained from various pharmaceutical enterprises of Ukraine during 2016-2018. The following equipment was used: Silica gel 60 $F_{254}$ TLC plates (20x20 $cm^2$, 0.25 mm), Merck (Germany); Ultrasonic bath SUPER RK100H «Bandelin», (Germany); Glass vertical chamber; Automatic spraying device ChromaJet DS20. As standard substances have been used alantolactone, isoalantolactone and dihydroisoalantolactone (<95%) - pharmacopoeial reference standards of the State Pharmacopoeia of Ukraine. **Results and discussion.** A procedure for identification of elecampane by TLC method for the national monograph of the SPhU "Elecampane roots and rhizomes" has been developed.. The identification method is based on the ability of silver ions to react with unsaturated C-C bonds in the molecules of the isomers at the position of double bonds, which are alantolactone and isoalantolactone. The development of a procedure for identification was carried out in conjunction with its validation in accordance with the requirements of SphU according to the following scheme: 1) the choice of stationary phase (examination of plates with a thin layer of silica gel treated with 1, 3, 5, 7% (m/v) solutions of silver nitrate); 2) the choice of mobile phase (review of unified phases for chromatography of terpenoids); 3) the choice of concentration for markers-substances (study of 0.05, 0.1, 0.2% methanol solutions of alantolactone, iso-alantolactone, dihydroiso-alantolactone and SQTL mixture for preparation of the reference solution); 4) the choice of the method for test solution preparation (study of extraction of herbal material with methanol, followed by concentration of the extract to the ratio of HD-test solution 1: 2, 1: 5, 1: 8); the choice of application volume of the test solution ; 5) the choice of the detection method (review of unified reagents and/or solutions for derivatization of chromatograms). Following conditions for identification have been chosen: the test solutions of HD (1: 5 in methanol), standard solution *CRS SPhU*

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alantholactone and isoalantholactone (0.1% solutions in methanol), TLC plates with a thin layer of silica gel treated with 5% silver nitrate solution, a solvent system toluene-ethyl acetate (9: 1), detection is carried out after treatment the plate with anise aldehyde solution and followed by heating. **Conclusion.** A procedure for identification of elecampane by for the national monograph of the SPhU "Elecampane roots and rhizomes" has been developed. It allows to identify such biologically active substances of the elecampane, as sesquiterpene lactones, which are markers of this species The developed chromatographic conditions allow to reliably chromatographically identify HD of elecampane in the presence on chromatograms of 3 zones of lactones – alantolactone, isoalantolactone and dihydroisoalantolactone.

**Key words**. Elecampane roots and rhizomes, sesquiterpene lactones, monograph of the SPhU, TLC method, alantolactone, isoalantolactone and dihydroisoalantolactone.

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